Features of post-operative immune suppression are reversible with interferon gamma and independent of IL-6 pathways

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Running head: Reversible perioperative immunosuppression and IL-6.

Abstract

Objective

To evaluate the role of IL-6 pathways in post-operative immune-suppression and to assess the reversibility of this phenomenon.

Background

The post-operative period is characterised by increased IL-6 production and features of immune-suppression. *In vitro*, IL-6 mediates anti-inflammatory effects through inhibition of interferon-gamma (IFN-γ) pathways. The significance of the immunomodulatory effects of IL-6 in the clinical setting of post-operative immune-suppression remains unclear.

Methods

Patients over 45 years old undergoing elective surgery involving the gastrointestinal tract were recruited. IL-6 levels were assayed using ELISA pre-operatively, at 24 and 48hrs. Peripheral blood mononuclear cells (PBMCs) from healthy volunteers were cultured in perioperative serum and $CD14^{+}$HLA-DR (mHLA-DR) geometric-mean florescent intensity (MFI) was measured in the presence and absence of IL-6 neutralising antibody and recombinant IFN-γ.

Results

Of 108 patients, 41 developed a post-operative infection. IL-6 levels increased 19-fold from the pre-operative sample to 24hrs post-operatively ($P<0.0001$). Higher IL-6 levels at 24 ($P=0.0002$) and 48hrs ($P=0.003$) were associated with subsequent post-operative infectious complications. mHLA-DR MFI fell when healthy PBMCs were cultured with post-operative serum compared to pre-operative serum ($P=0.008$). This decrease was prevented by the presence of IFN-γ in the culture media but not by the presence of IL-6 neutralising antibody.

Conclusions

IL-6 levels increase following major surgery and are associated with an increased susceptibility to post-operative infections. Serum obtained from post-operative patients induces an immunosuppressive response, reflected in reduced mHLA-DR levels, mediated through IL-6 independent pathways but reversible with IFN-γ. These data may have therapeutic implications for the prevention of infection in patients undergoing major surgery.

Introduction

Following major surgery patients experience a period of immune suppression, which can predispose to the development of post-operative infections.[1] Infectious complications result in considerable morbidity and are observed in over 30% of older patients undergoing major surgery.[2] Post-operative immune dysfunction is mediated partly by tissue injury and the consequent release of intracellular alarmins such as mitochondrial DNA fragments.[3] The cellular and transcriptomic features of tissue damage induced immune modulation are now well characterised.[4-6] Key reproducible cellular features of this response include a consistent fall in monocyte HLA-DR (mHLA-DR) expression[1, 7, 8] and an expansion of cells with phenotypic similarities to myeloid derived suppressor cells.[4, 5, 9] These alterations are quantitatively associated with infectious complications and poor functional recovery.[1, 4, 8] Although the immune suppressive effect of major tissue damage has been shown to be reversible with interferon gamma (IFN-γ) treatment in the context of traumatic injuries,[10-12] in the perioperative period the specific additional immunomodulating effects attributable to such factors as prolonged anaesthetic administration, opioid analgesics, premorbid conditions and blood transfusion require further consideration.[13-15]

There is similar consistency amongst the transcriptomic data available following tissue damage and major surgery.[6, 16, 17] A striking feature is the elevation in IL-6 levels in patients with features of functional immune suppression and the correlation between circulating levels of this cytokine and infectious complications. Although many essential pro-inflammatory effects are mediated through IL-6 pathways this cytokine has also been shown to drive differentiation of T helper cells to a T helper cell type 2 (T_h2) subtype and to inhibit T_h1 maturation through a Suppressor of Cytokine Signalling 1 (SOCS1) dependent suppression of IFN-γ signalling.[18, 19] These latter effects could be broadly construed as anti-inflammatory and may induce an immune environment in which host bactericidal abilities are impaired, thereby increasing susceptibility to infectious complications. Furthermore, the purported mechanism of action suggests that restoring IFN-γ activity may reverse the downstream consequences.

We hypothesised that increased levels of IL-6 following major surgery may promote an immunosuppressed phenotype that can be reversed with IFN-γ. In order to explore this hypothesis we aimed to describe the patterns of IL-6 production in response to major gastrointestinal surgery and the relationship between IL-6 levels and infectious complications. We also aimed to explore the role of IL-6 dependent pathways in post-operative immune suppression and to determine whether this immune suppression might be reversed by IFN-γ.

Methods

This study was conducted at The Royal London Hospital, London UK and approved by the North Wales Research Ethics Committee (Reference: 10/WNo03/25).

Patient selection

Consecutive patients aged over 45 years undergoing scheduled surgery involving the gastrointestinal tract, requiring a general anaesthetic and at least an overnight hospital stay were considered eligible for inclusion to this study. Exclusion criteria were refused consent, emergency surgery and surgery which also involved access to the thoracic cavity. Every patient on a weekday elective operating list was screened. Eligible patients were then approached for written informed consent. Entry into the study did not influence clinical treatment. All patients received perioperative prophylactic antibiotic therapy.

Data collection

Clinical data were collected on each patient until hospital discharge. Pre-operative immunosuppression was defined as the administration of chemotherapeutic agents and/or radiotherapy within the six months preceding surgery. Patients were examined daily by the clinical team for the presence of infection. Definitions of infection were agreed prospectively by the investigators and were based on the Center for Disease Control and Prevention (CDC) definitions and graded using the Clavien-Dindo classification (Supplementary Table 1).[20]

Blood sampling and processing

Blood samples were taken immediately before induction of anaesthesia (Pre-op), at 24 and 48 hours following the operation. Plasma was collected from citrated Vacutainer™ tubes (Becton Dickinson, UK) centrifuged twice at 3,000 RPM for 10 mins at 20^{o}C and stored at -80^{o}C until further analysis. Serum was collected from BD Vacutainer™ SST™ Serum Separation Tubes (Becton Dickinson, UK), allowed to clot for a minimum of 15 minutes and centrifuged at 3,000 RPM for 10 minutes at 20^{o}C and stored at -80^{o}C until further analysis. Serum was filtered with an Acrodisc® 32mm 1.2uM syringe filter (VWR, UK) prior to use.

Enzyme Linked Immunosorbent Assay (ELISA)

Plasma samples were assayed in duplicate using commercially available IL-6 ELISAs (Life Technologies, Carlsbad, CA) with absorbance measured at 450nm. We have previously attempted to quantify IFN-γ levels in plasma from similar patient groups and found levels to be unreliably detectible in the majority of patients; consequently we chose not to attempt to measure IFN-γ levels in this population.

In vitro cell culture experiments

8 mL of peripheral blood was collected in BD Vacutainer™ Sodium Citrate CPT™ tubes (Becton Dickinson, UK) from healthy controls (n=6, median age 36, male sex 4 (66.6%)). These were immediately centrifuged at 1,800 RCF for 30 minutes at 20^{o}C with the brake off. The peripheral blood mononuclear cell (PBMC) layer was isolated and washed in Phosphate Buffered saline (Life Technologies, Carlsbad, CA) containing 2% human albumin solution. Cells were then counted with a haemocytometer.

Pooled healthy PBMCs were aliquoted (3x10^{6} cells per well) into a 96 well plate and cultured in duplicate with Gibco® RPMI 1640 medium (Life Technologies, Carlsbad, CA) containing 30% patient serum, which was taken either pre-operatively (pre-op) or at 24hrs post operatively, for 20 hours at 37^{o}C

with 5% CO_2 (CB 150, Binder). The patients from whom perioperative serum was obtained were selected in order to have relatively homogenous operation times above a minimum time of 200 minutes (Supplementary Table 2 & 3).

Neutralising experiments: Pooled healthy PBMCs were pre-incubated with an Fc Block™ (Becton Dickinson, UK) for 30min then incubated with 30% 24hr serum and media pre-incubated with either IL-6 neutralising antibody 15ng/mL (AB-206-NA R&D systems, UK) for a minimum of 1 hour or with control non-specific goat IgG at 15ng/mL (AB-108-C, R&D systems, UK). These were cultured as described above.

IL-6 protein was quantified as above in four duplicate patient samples with and without 15ng/ml IL-6 neutralising antibody in order to demonstrate the efficacy of the neutralising effect (Supplementary figure 1).

Stimulating experiments: Pooled healthy PBMCs were incubated with 30% serum from either baseline or 24hrs with and without IFN-γ (250 International Units, R&D systems, UK). These were cultured as described above.

Flow Cytometry

Following culture, PBMCs were stained in duplicate for 30 minutes at room temperature with a combination Quantibrite™ antibody HLA-DR PE/anti Monocyte (CD14) and PerCP-Cy5.5 (Becton Dickinson, UK), cells were washed and immediately analysed. 2,000 $CD14^+$ cells were collected using an LSR II flow cytometer (Becton Dickinson, UK). $CD14^+$HLA-DR membrane density (geometric mean fluorescent intensity (MFI)) was analysed using FlowJo software 10.0.7 (Tree Star, Ashland, OR) on a MAC® workstation by gating on monocytes, via side and forward scatter, then gating on the $CD14^+$ population (Figure 1).

Statistical analysis

Results are expressed as median and interquartile range (IQR). All statistical tests are two-sided and a *P*-value of $P<0.05$ was considered significant. Differences in categorical variables were calculated using a Chi-squared or Fisher's exact test as appropriate. The Kruskal-Wallis test was used for continuous variables and the Wilcoxon signed-rank test for repeated measurements. Multivariable linear regression models were used to assess whether IL-6 levels were independently associated with post-operative infection. Data analysis was performed using the JMP (version 11) statistical software package (SAS, Cary, NC, USA).

Results

Patients

108 patients (mean age 65, range 46–87, 59% male) undergoing elective major abdominal surgery were included in this study. 41 (38%) patients developed a post-operative infection. Patient characteristics are outlined in Table 1.

Post-operative infections

Post-operative infectious complications developed a median of 9 (IQR 5–11) days following the procedure. The sites of infection and organisms isolated are shown in Table 2. Patients developing infections stayed longer in hospital (14 (9–19) vs 7 (5-9) days, $P<0.0001$). A range of demographic and clinical data did not distinguish between those who did and did not develop infection (Table 1).

Perioperative IL-6 levels

IL-6 levels increased 19-fold from baseline to 24 hours post-operatively ($P<0.0001$) and were unchanged between 24 and 48 hours post-operatively (Figure 2A). Baseline IL-6 levels were higher in patients with a diagnosis of cancer, $P=0.02$; Figure 2B). None of age, sex, smoking history, immunosuppression or diabetes was associated with baseline IL-6 levels. At 24 hours higher IL-6 levels were observed in patients who would subsequently develop infections ($P<0.0001$; Figure 3A). This association was observed for intra-abdominal infections ($P=0.004$; Figure 3B) and surgical site infections ($P=0.001$; Figure 3C) but not for pneumonia ($P=0.81$; Figure 3D). IL-6 levels at 24 hours were directly correlated with the length of the procedure (Spearman's ρ 0.26, $P=0.01$), were lower in patients who underwent endoscopic procedures (24 (12–47) vs 77 (32-136), $P=0.0001$) and were higher in patients that received an epidural (80 (31–172) vs 34 (18–84), $P=0.006$). No difference was detected in peri-operative IL-6 levels between those patients with and without pre-operative immunosuppression (Supplementary

Figure 2). A multivariable linear regression model confirmed that the association between IL-6 levels at 24 hours and post-operative infectious complications remained when corrected for the differences in the above variables. This pattern was replicated at 48 hours with IL-6 levels higher in those patients subsequently developing infections (P=0.003), higher in patients who had longer procedures (Spearman's ρ 0.26, P=0.02), lower in patients who had endoscopic procedures (P=0.0002) and higher in patients that received an epidural (P=0.02). The association between IL-6 levels at 48 hours and infectious complications again remained when corrected for these variables.

Effect of post-operative serum on healthy control mHLA-DR expression

Serum was collected from 8 patients pre-operatively and again at 24 hours. Details of these patients are given in Supplementary Table 2. Healthy control mHLA-DR MFI fell when incubated with serum collected 24 hours following major surgery when compared with MFI levels following incubation with pre-operative serum (Figure 4A, P=0.008, n=8). Pre-incubation with IL-6 neutralising antibody did not alter this reduction in MFI (Figure 4B; P=0.95). Serum from a further 8 patients was collected pre-operatively and at 24 hours (Supplementary Table 3). Again there was a fall in mHLA-DR MFI following incubation with serum taken at 24 hours. Co-incubation with IFN-γ resulted in an increase in mHLA-DR MFI (P=0.008; figure 4C, n=8). These data were also re-analysed excluding the two patients with pre-operative immunosuppression. A similar fall in mHLA-DR MFI following incubation with serum taken at 24 hours was demonstrated which was prevented by co-incubation with IFN-γ (n=6, supplementary figure 3A & B).

Discussion

In this study we determined plasma levels of IL-6 protein in patients over 45 years of age undergoing elective surgery involving the gastrointestinal tract and described an independent association between higher post-operative levels of IL-6 and later nosocomial infections. We also demonstrated that serum collected in the post-operative period contains soluble mediators, which reduce the antigen presenting capabilities of healthy monocytes as measured by HLA-DR expression. Although the causative molecules and pathways remain unidentified we report that IL-6 dependent pathways are not essential mediators of this immune suppressed phenotype. Treatment with IFN-γ *in vitro* did, however, reverse the induced deficit in the antigen presenting capabilities of monocytes exposed to post-operative serum.

The association between IL-6 levels and post-operative infections is not unexpected and has been previously described.[21, 22] Unsurprisingly, we also report that IL-6 levels are higher in those patients undergoing lengthier procedures, which are likely to be more complex with a higher probability of post-operative complications. Importantly, however, we have found that the association between IL-6 levels and post-operative infections is independent of the duration of the surgical procedure. It is also well recognised that post-operative patients display features of immune suppression.[1, 7] However, whereas previous investigators have reported alterations in the properties of specific cell subtypes or their cell surface markers[4, 7] we demonstrate that immune suppression is mediated, at least in part, by soluble compounds that remain 24 hours following surgery, as opposed to the direct effect of either tissue damage, anaesthesia or endotoxin release on immune cell subtypes. These data may be of crucial importance in developing potential treatments to alleviate post-operative immune suppression.

The key link between the tissue damage characteristic of major surgery and the subsequent inflammatory response is the release of alarmins.[23] Alarmins are a group of structurally diverse compounds, which include high-mobility-group box (HMGB) proteins and mitochondrial DNA (mtDNA), which are released following tissue damage as cells undergo physiological stress or necrosis.[24] These are then recognized by a wide variety of pattern recognition receptors (PRRs), which include the

membrane bound Toll-like receptors (TLRs) and the cytoplasmic NOD-like receptors (NLRs).[24] Activation of PRRs induces an enzymatic cascade, which results in down-stream phosphorylation of transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which in turn alters cytokine transcription. IL-6 is a cytokine that is consistently upregulated acutely following activation of this cascade.[6] Furthermore, following traumatic injury elevated levels of IL-6 are seen concurrently with evidence of immune suppression and reduced antigen presentation capabilities.[25] This association, together with the consistent observation that higher IL-6 levels and infectious complications are linked following major tissue damage raises the possibility that in this scenario IL-6 functions not in its traditional pro-inflammatory role but rather to promote an immune suppressed phenotype. This hypothesis is supported by evidence from animal models and *in vitro* data describing the anti-inflammatory effects of IL-6 acting through a classical signaling pathway involving the membrane bound IL-6 receptor and SOCS1 and STAT3 dependent mechanisms which ultimately inhibit IFN-γ production.[18, 19, 26] Although IL-6 antagonists such as tocilizumab are in clinical use, it is imperative that prior to considering their potential use following major surgery evidence is available that the increased IL-6 observed in this scenario is of significance as a specific mediator of poor clinical outcomes as opposed to being a biomarker of a poor clinical outcome.[27] In the case of post-operative immune suppression our data suggests that although elevated IL-6 levels are associated with clinical correlates of immune suppression such as post-operative infectious complications it does not play a key role in impairing the antigen presenting capabilities in the post-operative period. Interestingly, it has recently been reported that the development of post-operative inflammatory complications and infection in particular, is preceded by increased expression of TLR4 and TLR5 on an intermediate ($CD14^{++}CD16^{+}$) monocyte population.[28] These same patients displayed higher resting and TLR-stimulated IL-6 levels in the early post-operative period in conjunction with enhanced activity in the NF-κB signaling pathway.[28]

We further hypothesised that were IL-6 to play a major role in mediating post-operative immune suppression through a pathway that culminates in IFN-γ inhibition[19] then supplementation with exogenous IFN-γ may reverse these features. Although it appears that the increase in IL-6 levels may not

play a causal role in this process our results indicate that IFN-γ independently restores HLA-DR expression in affected monocytes. There are conflicting reports in the surgical literature on the effect of post-operative IFN-γ administration. In patients with colonic cancer IFN-γ appears to increase HLA-DR expression on PBMCs,[29] whereas in other patients undergoing gastrointestinal surgery IFN-γ administered as part of *in vitro* experiments failed to reconstitute defective pro-inflammatory cytokine production.[29] Our data demonstrates that the *in vitro* administration of IFN-γ can reverse features of defective antigen presenting capacity following major surgery in patients both with and without cancer. Our use of a well-validated, standardized biomarker of immune suppression (mHLA-DR) of particular relevance in the prediction of septic complications enhances the potential clinical applicability of these results.[25, 30, 31]

There are a number of inherent strengths to our study. We have studied a clinically relevant at-risk population selected by using age over 45 years old as an inclusion criterion. This ensures that the event rate of our primary outcome, infectious complications following elective surgery, is sufficiently high at 38% and is in keeping with recent data from similar cohorts.[2] As all patients were undergoing elective surgery this allows each patient in the *in vitro* study to act as their own control. This is important as a significant number of these patients had a diagnosis of cancer or were immunocompromised and the use of healthy control serum as a comparator would be inappropriate. Finally, we describe a relatively novel method of identifying perioperative immune suppression by culturing healthy monocytes in post-operative serum in conjunction with the well-validated methodology of defining immune suppression by observing a decrease in mHLA-DR expression.[31, 32]

Limitations of this study include; not assaying circulating IFN-γ protein levels in the post-operative period and the wide variability in the surgical procedures performed resulting in a heterogeneous patient population. We chose not to assay circulating IFN-γ protein levels due to the insensitivity of the IFN-γ assay in clinical conditions where in our experience IFN-γ levels are frequently undetectable.

Conclusions

IL-6 levels increase following major surgery and higher levels are associated with an increased susceptibility to post-operative infections. Serum obtained from post-operative patients induces an immunosuppressive response through IL-6 independent pathways, which is reversible with IFN-γ administration. Although, increased IL-6 levels may be useful as a biomarker of impending infectious complications our data do not support a causal relationship.

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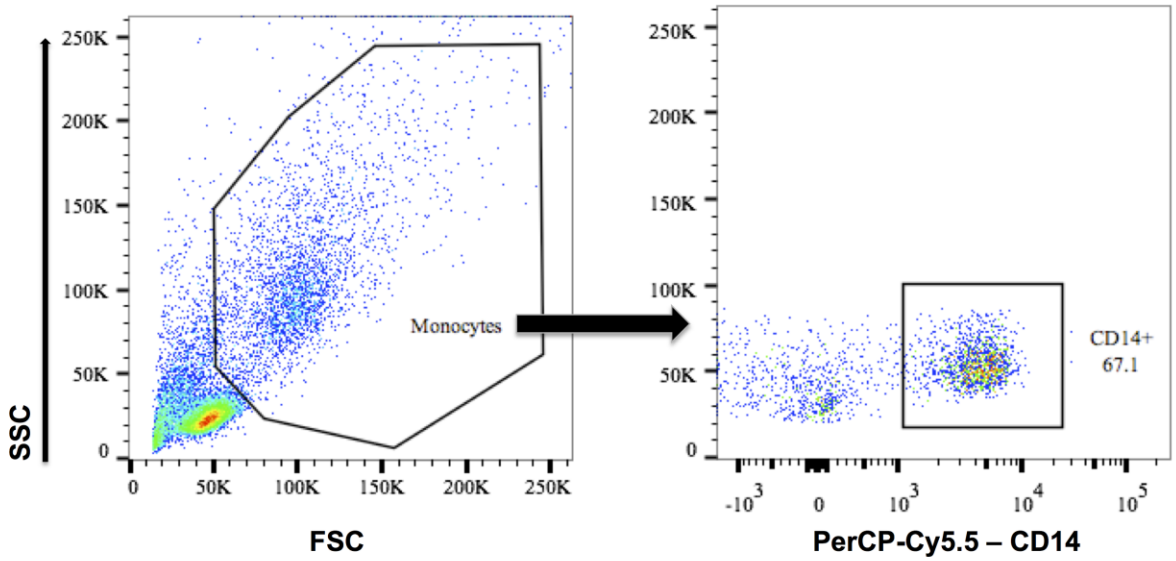


Figure 1: Representative flow cytometry plot of the gating strategy.

Cells were gated according to their side and forward scatter properties in order to identify the monocyte subpopulation. This subpopulation was then gated according to CD14 expression. $CD14^+$ cells were then analysed for HLA-DR geometric mean intensity (MFI).

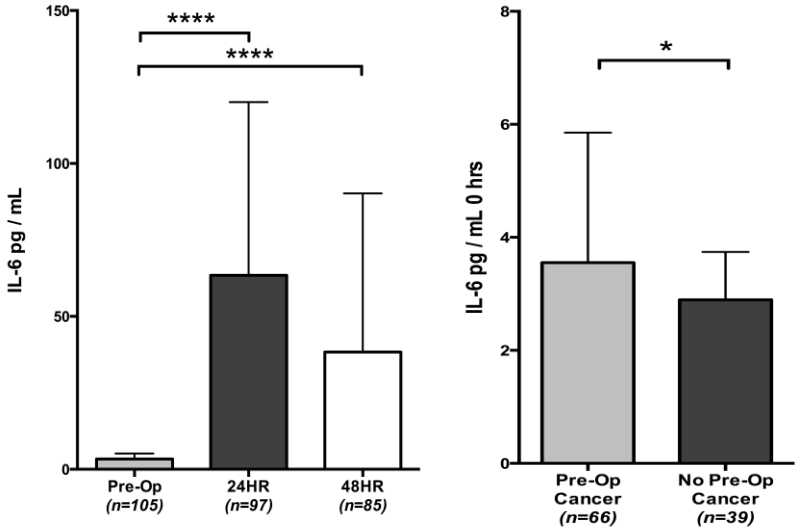


Figure 2: IL-6 plasma levels, the influence of surgery and pre-operative cancer.

A: Changes in IL-6 assayed IL-6 plasma concentration following major gastrointestinal surgery. **B**: Pre-operative IL-6 protein levels were raised in patients who had a diagnosis of cancer. All data displayed as median and interquartile range, pg = picograms, *P<0.05 *, P≤0.0001 ****.*

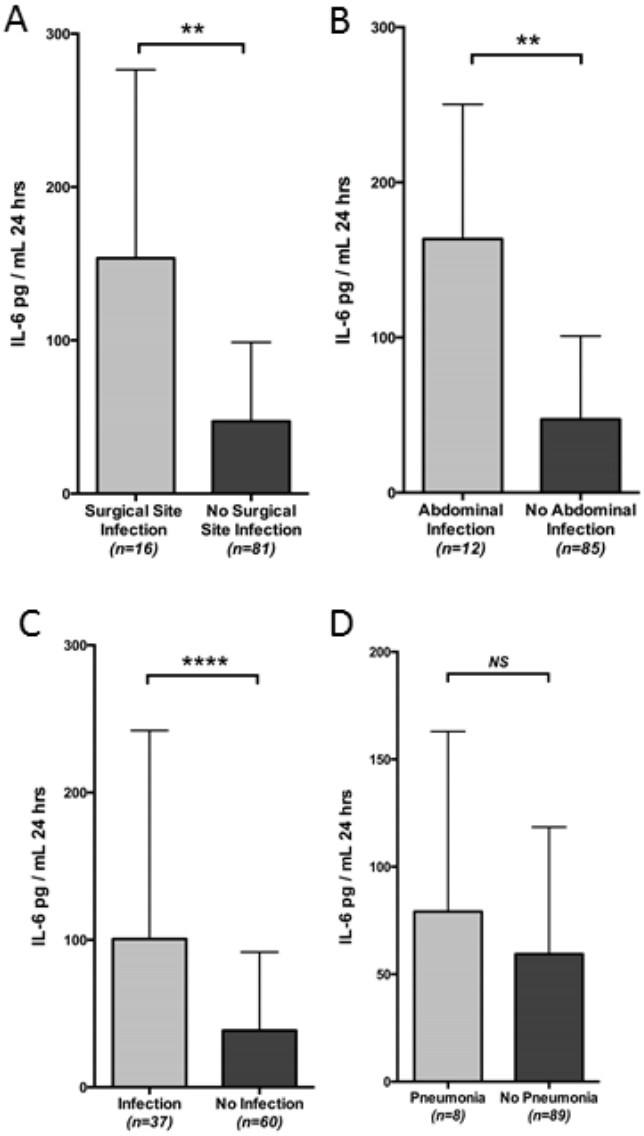


Figure 3: Post-operative IL-6 levels and infectious complications.

IL-6 concentration assayed at 24 hours between those patients suffering any post-operative infection (**A**), a post-operative abdominal infection (**B**), a post-operative surgical site infection (**C**) or a post-operative pneumonia (**D**). Infections were defined according to CDC criteria.[20] All data displayed as median and interquartile range, *P≤0.01 **, P≤0.0001 ****

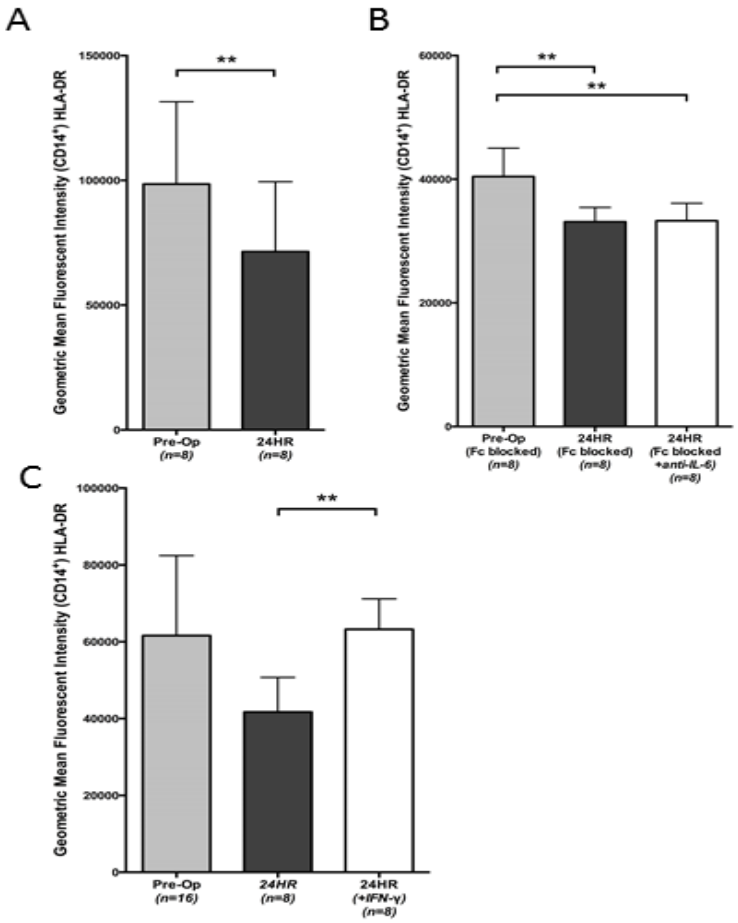
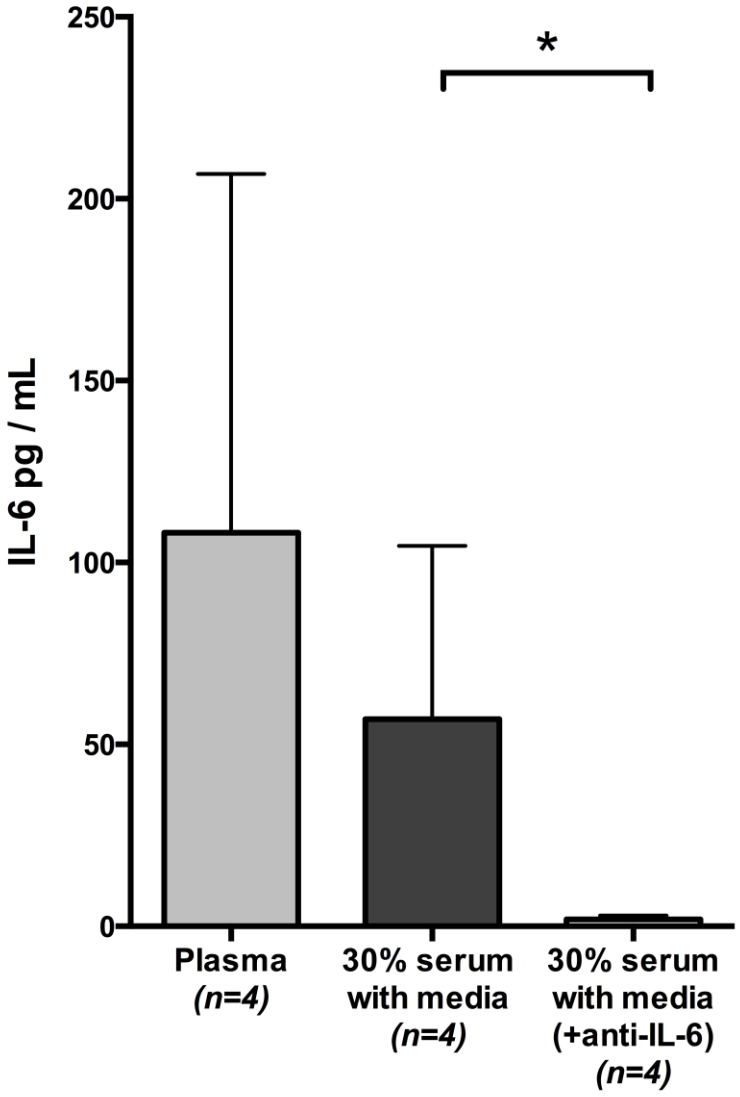
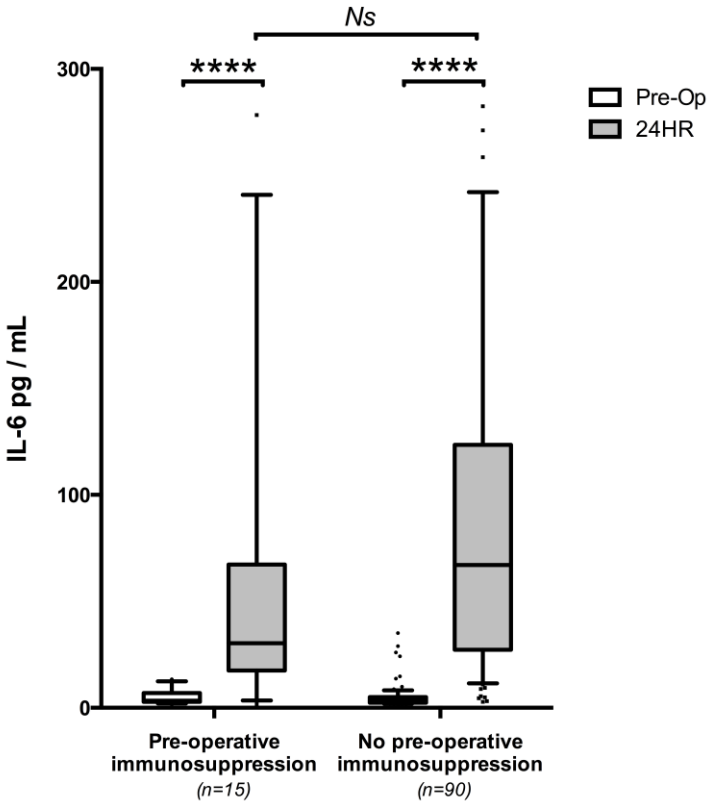


Figure 4: Perioperative serum, healthy PBMCs and the effects of IL-6 neutralisation and IFN-γ stimulation. A: Post-operative serum decreases the level of mHLA-DR on cultured PBMCs obtained from healthy controls. **B:** The addition of an Fc blocker to the culture medium was necessary to eliminate nonspecific antibody actions. In this experiment, in the presence of an Fc blocker, post-operative serum similarly induced a fall in healthy PBMC mHLA-DR levels. This fall in mHLA-DR levels was unchanged when IL-6 was neutralised by the addition of IL-6 antibody at 15ng/ml. **C**: In comparison, when post-operative serum was co-cultured with IFN-γ (250 IU) mHLA-DR levels were restored to those levels seen with pre-operative serum. All data (**A-C**) from two independent experiments. All data displayed as median and interquartile range, HLA-DR quantified as geometric median fluorescence intensity (MFI) on $CD14^+$ cells, $P \leq 0.01$ **.



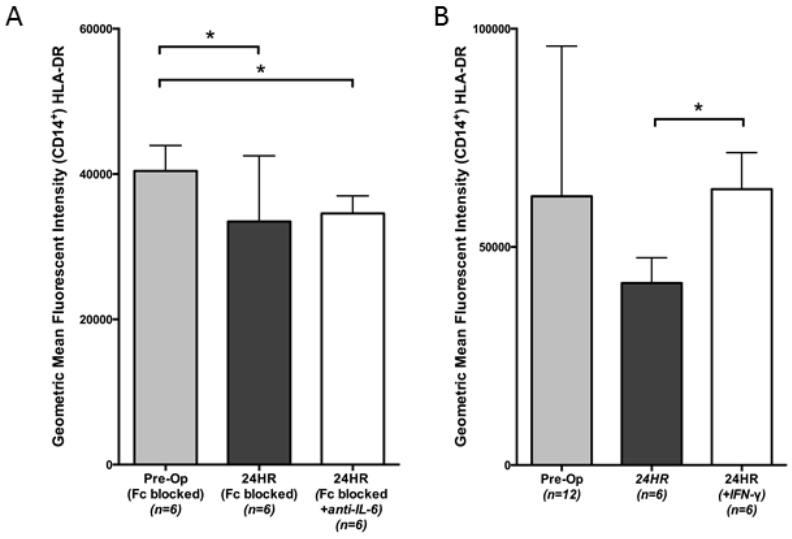
Supplementary Figure 1: Efficacy of the IL-6 blocking antibody

A: Changes in IL-6 protein concentration when post-operative serum was cultured in the presence or absence of an IL-6 blocking antibody. All data displayed as median and interquartile range, *P<0.05 **.



Supplementary Figure 2: Perioperative plasma IL-6 levels in patients with and without pre-operative immunosuppression

A: Changes in IL-6 protein concentration assayed pre-operatively and at 24 hours post-operatively in patients with and without pre-operative immunosuppression. All data displayed as median and interquartile ranges, *P<0.0001* ****.



Supplementary Figure 3: Perioperative serum, healthy PBMCs and the effects of IL-6 neutralisation and IFN-γ stimulation in patients without pre-operative immunosuppression

A: The addition of an Fc blocker to the culture medium was necessary to eliminate nonspecific antibody actions. In this experiment, in the presence of an Fc blocker, post-operative serum similarly induced a fall in healthy PBMC mHLA-DR levels. This fall in mHLA-DR levels was unchanged when IL-6 was neutralised by the addition of IL-6 antibody at 15ng/ml. **B**: In comparison, when post-operative serum was co-cultured with IFN-γ (250 IU) mHLA-DR levels were restored to those levels seen with pre-operative serum. All data (**A-B**) from two independent experiments. All data displayed as median and interquartile range, HLA-DR quantified as geometric median fluorescence intensity (MFI) on $CD14^+$ cells, *P<0.05 *.*

Supplementary Table 1. Criteria used for defining the sites of infection.[19]

Infection site	Definition
BSI – Bloodsteam Infection (LCBI - Laboratory-Confirmed Bloodstream Infection, Secondary BSI- Secondary Bloodstream Infection)	**Laboratory-Confirmed Bloodstream Infection (LCBI)** must meet at least 1 of the following criteria: 1. Patient has a recognized pathogen cultured from 1 or more blood cultures. **AND** organism cultured from blood is not related to an infection at another site. 2. Patient has at least 1 of the following signs or symptoms: fever (>38°C), chills, or hypotension. **AND** signs and symptoms and positive laboratory results are not related to an infection at another site. **AND** common skin contaminant is cultured from 2 or more blood cultures drawn on separate occasions. **Secondary Bloodstream Infection (BSI)** In a patient suspected of having an infection, blood and a site-specific specimen are collected for culture and both are positive for at least one matching organism. If the site-specific culture is an element used to meet the infection site criterion, then the BSI is considered secondary to that site-specific infection.

Supplementary Table 2: Anti-IL-6 Cell culture patient sample demographics

Male	8 (100)
Age (years)	64 (58 – 74)
ASA grade 3 or 4	1 (12.5%)
Operation details	
Elective (%)	8 (100)
Planned postoperative ICU admission	8 (100)
Operation length (minutes)	296 (255 – 326)
Colorectal	2 (25%)
Upper GI	2 (25%)
HPB	4 (50%)
Laparoscopic	2 (25%)
Cancer	5 (62.5%)
Preoperatively immunosuppressed	2 (25%)
Nosocomial Infection	3 (37.5%)
Wound	2 (25%)
UTI	1 (12.5%)
Days post op infection (days)	11, (2-14)
Plasma IL-6 (ELISA) (pg/ml)	
Pre-Op	2.47 (2.25 – 3.08)
24hrs	64.91 (41.17 – 93.36)
48hrs	79.96 (30.33 – 86.11)

Data are described as medians with interquartile range or numbers with percentages in parenthesis. ICU, Intensive Care Unit. GI, Gasto-Intestinal. HPB, Hepato-Pancreato-Biliary.

Supplementary Table 3: IFN-γ Cell culture patient sample demographics

Male	5 (62.25%)
Age (years)	60 (52 – 69)
ASA grade 3 or 4	1 (12.5%)
Operation details	
Elective	8 (100%)
Planned postoperative ICU admission	8 (100%)
Operation length (minutes)	286 (225 – 362)
Colorectal	3 (37.5%)
Upper GI	2 (25%)
HPB	3 (37.5%)
Laparoscopic	3 (37.5%)
Cancer	6 (75%)
Preoperatively immunosuppressed	1 (12.5%)
Nosocomial Infection	2 (25%)
Wound	1 (12.5%)
UTI	2 (25%)
Pneumonia	1 (12.5%)
Intra abdominal	1 (12.5%)
Days post op infection (days)	15.5 (8 – 24)
Plasma IL-6 (ELISA) (pg/ml)	
Pre-Op	2.74 (2.08 – 3.33)
24hrs	47.31(27.02 – 176.90)
48hrs	25.88 (16.68 – 58.63)

Data are described as medians with interquartile range or numbers with percentages in parenthesis. ICU, Intensive Care Unit. GI, Gasto-Intestinal. HPB, Hepato-Pancreato-Biliary.